PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Tatsuo KAKIMOTO, et al.

Docket No: Q65478

Appln. No.: 09/918,508

Group Art Unit: 1647

Confirmation No.: 3296

Examiner: Cherie Michelle WOODWARD

Filed: August 01, 2001

For: ANALYSIS OF AGONIST-ACTIVITY AND ANTAGONIST-ACTIVITY TO CYTOKININ RECEPTOR

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, TATSUO KAKIMOTO do hereby declare and state:

THAT I am a co-inventor of the subject matter disclosed and claimed in the above-mentioned application;

THAT I am a co-author of *Nature* Vol. 409, 1060-1063 (2001); and

THAT the present invention was invented prior to October 16, 2000, as evidenced by the date that the manuscript published as *Nature* Vol. 409, 1060-1063 was received by the Journal Nature for publication.

THAT the attached email dated November 14, 2000, was sent to me (at the email address kakimoto@bio.sci.osaka-u.ac.jp) from Chris Surridge (at the email address C.Surridge@nature.com), the Senior Editor of the journal *Nature*.

THAT the email contains comments of the editor and two referees and does not contain any comments relating to substantive changes to the subject matter of the article.

THAT the comments from the editor and the two referees show that my co-inventors and I had possession of the claimed invention before November 29, 2000.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: _____ Name: _____
TATSUO KAKIMOTO

>> From: "Surridge, Chris" <C.Surridge@nature.com>
>> To: "'kakimoto@bio.sci.osaka-u.ac.jp'"
>> <kakimoto@bio.sci.osaka-u.ac.jp>
>> Subject: Nature manuscript K10294
>> Date: Tue, 14 Nov 2000 11:18:06 -0000
>> MIME-Version: 1.0
>> Status:
>>
>> In reply please quote:
>> K10294 CS/sad
>>
>>
>>
>> Dear Dr Kakimoto,
>> Your manuscript "Identification of a cytokinin receptor, CRE1, from
>> Arabidopsis" has now been seen by our two referees, whose comments are
>> attached. In the light of their advice we shall be happy, in
>> principle, to
>> publish it in Nature. First, however, we must ask you to address the
>> points
>> raised by our referees and ensure that it complies with the
>> requirements set
>> out in our Extended Guide to Authors at http://www.nature.com/submit/.
>> To ensure the accessibility of your paper to readers from other
>> disciplines,
>> we ask you to pay particular attention to the wording of the paper's
>> opening
>> paragraph, which should serve both as an introduction and as a brief,
>> non-technical summary. It should start by outlining the background to
>> the
>> present work, before going on to describe your new observations and
>> main
>> conclusions. As it is part of the main text, it should cite
>> references as
>> appropriate, and its contents should not be repeated elsewhere.
>> Because we

>> hope that all biologists will be interested in your results and their
>> implications, it is important to explain essential but specialized
>> terms
>> concisely. It is almost invariably worthwhile to show your
>> introduction to
>> colleagues specializing in other areas to uncover any problematic
>> concepts.
>> We have also found that it is best to avoid using the passive voice or
>> sentences with more than one relative clause wherever possible.
>> Your manuscript is also a little longer than our optimum for Letters
>> to
>> Nature. With this in mind it seems to us that it is not essential to
>> present
>> figure 3 in the paper, the results being adequately presented in the
>> text.
>> please therefore either delete figure 3 completely or move it to your
>> supplementary information.
>> Where possible, we would be grateful for production-quality copies of
>> your
>> figures on disk; please follow the guidelines at
>> http://www.nature.com/submit/ when preparing them. We would also be
>> grateful
>> for a copy of the text on a floppy disk to assist our subeditors as
>> well as
>> a separate disk containing your supplementary information and hardcopy
>> thereof. To avoid unwieldy titles, we ask that they fit into three
>> lines of
>> 31 characters each, and do not contain punctuation; we suggest
>> "Identification of CRE1 as a cytokinin receptor from Arabidopsis".
>> Finally,
>> it is a condition of publication that you include a statement before
>> the
>> acknowledgements naming the author to whom correspondence and
>> requests for
>> materials should be addressed.
>> We hope to receive your revised manuscript, of which we shall need

>> three
>> copies, within the next three weeks; please let us know if the delay
>> is
>> likely to be longer. As soon as we have the final text we shall
>> proceed with
>> rapid publication.
>> Yours sincerely,
>>
>>
>> Christopher Surridge
>> Senior Editor
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>> ----------
>> Ref 1
>> Review: Identification of a cytokinin receptor, CRE1, from
>> Arabidopsis by T
>> Inoue, M Higuchi, Y Hashimoto et al.
>> This paper is a logical and focused development of Kakimoto's earlier
>> work
>> (published in Science (1996) 274, 982) on a cytokinin response gene
>> that
>> encoded a histidine kinase. At that stage there was neither genetic
>> nor
>> biochemical data to show that CKI1, the gene in question, was a
>> cytokinin
>> receptor. This new work shows convincingly that a cytokinin receptor,
>> like
>> the ethylene receptors, is a receptor histidine kinase that operates
>> through
>> a phosphotransfer relay as in bacterial two-component systems.
>> The group start by conducting a (labour intensive) EMS screen to
>> identify an
>> Arabidopsis mutant with reduced responses to cytokinin. The gene
>> responsible
>> (CRE1) is cloned and characterized and a second allele isolated using

>> a
>> directed screen of an insertional mutant collection. Both mutations
>> are
>> semi-dominant with respect to cytokinin sensitivity. Whether the CRE1
>> histidine kinase is a cytokinin receptor, was tested using a yeast
>> system
>> deficient in the osmosensor SLN1 histidine kinase. This confirmed
>> that CRE1
>> is indeed a receptor and that cytokinins are the functional ligands.
>> Furthermore site-directed mutagenesis experiments on CRE1 in yeast
>> confirmed
>> that the phosphorelay system (involving a histidine and aspartic acid
>> residue) are likely to be involved in the cytokinin signal
>> transduction
>> pathway.
>> The work is well conducted with appropriate controls and finally
>> provides
>> the breakthrough in cytokinin research the field has been waiting for.
>> Indeed this now begins to support a plant-specific paradigm in which
>> osmosensing, ethylene signalling and now cytokinin signalling operate
>> through histidine kinase-coupled receptors. This work has general
>> relevance
>> to the signalling field as well as enormous significance to the plant
>> 'hormone', or growth factor community.
>>
>> I recommend publication.
>>
>> Minor points:
>> 1. cDNA sequence accession number not yet supplied.
>> 2. Comment could have been made on the alternatively spliced messages,
>> and whether (and where) they are expressed
>> 3. Have Northern analyses confirmed that CRE1 is expressed in the
>> tissues used here
>> 4. Some comment is needed on the relation of CRE1 to their earlier
>> gene CKI1. Is this one of the two related genes with 'high sequence
>> similarity' mentioned in the text? Have they looked at the genetic

>> interactions between CRE1 and CKI1?
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>> Ref 2
>> This very exciting manuscript describes the isolation and
>> characterization
>> of a candidate cytokinin receptor in Arabidopsis. The phenotype of
>> the cre1
>> mutants strongly suggests that CRE1 is required for cytokinin
>> response. In
>> addition the ability of CRE1 to confer cytokinin-dependent growth in
>> the
>> sln1 mutant of yeast is a striking result. I think they really do
>> have a
>> receptor and I recommend publication. There are a number of
>> grammatical
>> errors that should be attended to. In addition, the authors may want
>> to
>> consider the following points.
>> page 2: a few words about how the mutants were isolated should be
>> included
>> here in addition to the methods section.
>> page 3: Fig 4 is mentioned in the text before Fig 3. The order of
>> these two
>> Figs should be reversed.
>> page 3: Did CRE1 also restore cytokinin sensitivity in the roots of
>> seedlings.
>> page 3: What is the origin of cre1-2? Was this line generated in the
>> authors' lab or is in a publicly available population.
>> page4: The cre mutants are not insensitive to cytokinin, this implies
>> no
>> cytokinin response. Cytokinin resistant is better.
>> page 4: More information should be provided about the two related
>> genes.

>> What are the accession numbers and how related are the proteins.
>> page 4: Apart form normal stature, were the mutants like wild-type in
>> other
>> respects?
>> page 4 and 5: It would be interesting to know the level of identity
>> between
>> CRE1 and SLN1. It is a bit surprising that CRE1 can interact with YPD.
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>> ------
>> ----------
>> In reply please quote:
>> K10294 CS/sad
>>
>> 14 November 2000
>>
>> Dr T Kakimoto
>> Department of Biology
>> Graduate School of Science
>> Osaka University
>> Machikaneyama 1-1, Toyonaka
>> Osaka 560-0043
>> Japan
>>
>>
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